



**NAVFAC**  
Naval Facilities Engineering Command

**ENGINEERING SERVICE CENTER**  
Port Hueneme, California 93043-4370

## **CONTRACT REPORT**

### **CR 05-005-ENV**

## **COST AND PERFORMANCE REPORT FOR BIOAVAILABLE FERRIC IRON (BAFeIII) ASSAY**

by

Carmen Lebron (PI), NFESC  
Patrick Evans, Ph.D. (Co-PI), CDM  
Mary Trute, CDM  
Roger Olsen, Ph.D., CDM  
Rick Chappell, Ph.D., CDM  
John Wilson, Ph.D., EPA/Ada  
Cherri Adair, EPA/Ada  
Eric Weber, Ph.D., EPA/Athens  
John Kenneke, Ph.D. EPA/Athens  
B.T. Thomas, Ph.D., EPA/Athens  
Tom DiChristina, Ph.D., GIT  
John Drexler, Ph.D., UC

June 2005

---

Approved for public release; distribution is unlimited.

| <b>REPORT DOCUMENTATION PAGE</b>   |             |                                |                                   | <i>Form Approved</i><br><i>OMB No. 0704-0811</i>                     |  |
|--|-------------|--------------------------------|-----------------------------------|--|--|
| <p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information, if it does not display a currently valid OMB control number.</p> <p><b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b></p> |             |                                |                                   |  |  |
| <b>1. REPORT DATE (DD-MM-YYYY)</b><br>June 2005  |             | <b>2. REPORT TYPE</b><br>Final |                                   | <b>3. DATES COVERED (From – To)</b><br>N/A                           |  |
| <b>4. TITLE AND SUBTITLE</b><br><b>COST AND PERFORMANCE REPORT FOR BIOAVAILABLE FERRIC IRON (BAFeIII) ASSAY</b>  |             |                                |                                   | <b>5a. CONTRACT NUMBER</b>   |  |
|  |             |                                |                                   | <b>5b. GRANT NUMBER</b>  |  |
|  |             |                                |                                   | <b>5c. PROGRAM ELEMENT NUMBER</b>                                    |  |
| <b>6. AUTHOR(S)</b><br>Carmen Lebron (PI), NFESC; Patrick Evans, Ph.D. (Co-PI), CDM; Mary Trute, CDM; Roger Olsen, Ph.D., CDM; Rick Chappell, Ph.D., CDM; John Wilson, Ph.D., EPA/Ada; Cherri Adair, EPA/Ada; Eric Weber, Ph.D., EPA/Athens; John Kenneke, Ph.D. EPA/Athens; B.T. Thomas, Ph.D., EPA/Athens; Tom DiChristina, Ph.D., GIT; John Drexler, Ph.D., UC  |             |                                |                                   | <b>5d. PROJECT NUMBER</b>  |  |
|  |             |                                |                                   | <b>5e. TASK NUMBER</b>   |  |
|  |             |                                |                                   | <b>5f. WORK UNIT NUMBER</b>  |  |
| <b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESSES</b><br>Commanding Officer<br>Naval Facilities Engineering Service Center<br>1100 23 <sup>rd</sup> Ave<br>Port Hueneme, CA 93043-   |             |                                |                                   | <b>8. PERFORMING ORGANIZATION REPORT NUMBER</b><br><br>CR-05-005-ENV |  |
| <b>9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b><br>Environmental Security Technology Certification Program<br>901 North Stuart Street, Suite 303<br>Arlington, VA 22203   |             |                                |                                   | <b>10. SPONSOR/MONITORS ACRONYM(S)</b><br>ESTCP                      |  |
|  |             |                                |                                   | <b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>                        |  |
| <b>12. DISTRIBUTION/AVAILABILITY STATEMENT</b><br>Approved for public release; distribution is unlimited.  |             |                                |                                   |  |  |
| <b>13. SUPPLEMENTARY NOTES</b>   |             |                                |                                   |  |  |
| <b>14. ABSTRACT</b><br>This report describes the demonstration and validation of a novel analytical technology: a bioavailable ferric iron (BAFeIII) assay. Demonstration and validation of the BAFeIII assay was conducted at four Department of Defense (DoD) installations.   |             |                                |                                   |  |  |
| <b>15. SUBJECT TERMS</b><br><br>Bioavailable ferric iron (BAFeIII) assay, soil, sediment, Environmental Security Technology Certification Program (ESTCP),   |             |                                |                                   |  |  |
| <b>16. SECURITY CLASSIFICATION OF:</b>   |             |                                | <b>17. LIMITATION OF ABSTRACT</b> | <b>18. NUMBER OF PAGES</b>   | <b>19a. NAME OF RESPONSIBLE PERSON</b>           |
| a. REPORT  | b. ABSTRACT | c. THIS PAGE                   |                                   |  | <b>19b. TELEPHONE NUMBER (include area code)</b> |
| U  | U           | U                              | U                                 | 42   |  |

# Environmental Security Technology Certification Program (ESTCP)

## Cost and Performance Report Bioavailable Ferric Iron (BAFeIII) Assay ESTCP Project Number CU-0009



**June 2005**

## Table of Contents

|  |    |
|--|----|
| 1.0 Executive Summary .....                                | 1  |
| 1.1 Background .....                                       | 1  |
| 1.2 Objectives of the Demonstration .....                  | 1  |
| 1.3 Regulatory Drivers .....                               | 2  |
| 1.4 Demonstration Results .....                            | 2  |
| 1.5 Stakeholder/End-User Issues .....                      | 2  |
| 2.0 Technology Description .....                           | 3  |
| 2.1 Technology Development and Application .....           | 3  |
| 2.2 Process Description .....                              | 4  |
| 2.3 Previous Testing of the Technology .....               | 6  |
| 2.4 Advantages and Limitations of the Technology .....     | 6  |
| 3.0 Demonstration Design .....                             | 10 |
| 3.1 Performance Objectives .....                           | 10 |
| 3.2 Selection of Test Sites .....                          | 10 |
| 3.3 Test Site History/Characteristics .....                | 11 |
| 3.4 Physical Set-up and Operations .....                   | 14 |
| 3.5 Sampling/Monitoring Procedures .....                   | 14 |
| 3.6 Analytical Procedures .....                            | 14 |
| 4.0 Performance Assessment .....                           | 16 |
| 4.1 Performance Data .....                                 | 16 |
| 4.2 Performance Criteria .....                             | 19 |
| 4.3 Data Assessment .....                                  | 21 |
| 4.4 Technology Comparison .....                            | 21 |
| 5.0 Cost Assessment .....                                  | 22 |
| 5.1 Cost Reporting .....                                   | 22 |
| 5.2 Cost Analysis .....                                    | 23 |
| 5.3 Cost Comparison .....                                  | 25 |
| 6.0 Implementation Issues .....                            | 26 |
| 6.1 Cost Observations .....                                | 26 |
| 6.2 Performance Observations .....                         | 26 |
| 6.3 Scale-Up .....   | 26 |
| 6.4 Other Significant Observations .....                   | 26 |
| 6.5 Lessons Learned .....                                  | 27 |
| 6.6 End-User Issues .....                                  | 27 |
| 6.7 Approach to Regulatory Compliance and Acceptance ..... | 27 |
| 7.0 References .....                                       | 28 |
| 8.0 Points of Contact .....                                | 31 |
| 8.1 ESTCP Program Office .....                             | 31 |
| 8.2 Project Management .....                               | 31 |
| 8.3 CDM Staff .....  | 31 |
| 8.4 Partners .....   | 32 |

## List of Figures

|     |   |    |
|-----|---|----|
| 2-1 | BAFeIII Assay Kit.....  | 3  |
| 2-2 | BAFeIII Assay Kit Procedure.....  | 5  |
| 3-1 | Soil Sample Allocation and Analysis .....                                 | 15 |
| 4-1 | Intra-Laboratory Replicate Precision for BAF <sub>e</sub> III Assay ..... | 16 |
| 4-2 | Inter-Laboratory Replicate Precision for BAF <sub>e</sub> III Assay ..... | 17 |
| 4-3 | Summary of Iron Oxide Analytical Results.....                             | 18 |

## **List of Tables**

|     |  |    |
|-----|--|----|
| 1-1 | Performance Objectives and Results for the BAF <sub>FeIII</sub> Assay.....                     | 2  |
| 2-1 | Advantages and Limitations of the BAF <sub>FeIII</sub> Assay.....                              | 6  |
| 2-2 | Other Methods for BAF <sub>FeIII</sub> Measurement .....                                       | 8  |
| 3-1 | Performance Objectives for BAF <sub>FeIII</sub> Assay .....                                    | 10 |
| 4-1 | Component Loadings.....  | 17 |
| 4-2 | Iron Mass Balance for Elizabeth City Fuel Farm .....   | 19 |
| 4-3 | Expected Performance and Performance Confirmation Methods for BAF <sub>FeIII</sub> Assay ..... | 19 |
| 5-1 | BAF <sub>FeIII</sub> Assay Costs .....   | 23 |
| 5-2 | BAF <sub>FeIII</sub> Cost Scenarios .....  | 24 |

## Acronyms

|                   |   |
|-------------------|---|
| AFB               | Air Force Base  |
| AHDS              | Anthraquinol disulfonate  |
| AO                | Ammonium oxalate  |
| AQDS              | Anthraquinone disulfonate   |
| ASTM              | American Society for Testing Materials  |
| AOFE              | Ammonium oxalate extractable iron   |
| BAFeIII           | Bioavailable ferric iron  |
| BET               | Brunauer-Emmett-Teller  |
| bgs               | Below ground surface  |
| BTEX              | Benzene-toluene-ethylbenzene-xylenes  |
| BrY               | <i>Shewanella alga</i> BrY  |
| CDB               | Citrate dithionite bicarbonate  |
| CDBFE             | Citrate dithionite bicarbonate extractable iron   |
| cDCE              | <i>cis</i> -Dichloroethene  |
| CDM               | Camp Dresser & McKee Inc.   |
| CDMBAFeIII        | Bioavailable ferric iron measured by CDM  |
| C <sub>E</sub>    | Extract concentration   |
| CFR               | Code of Federal Regulations   |
| DCE               | Dichloroethene  |
| DoD               | U.S. Department of Defense  |
| EAB               | Enhanced anaerobic biodegradation   |
| EGDY              | East Gate Disposal Yard   |
| EPA/Ada           | U.S. Environmental Protection Agency, Subsurface Remediation Division,<br>National Risk Management Research Laboratory, Ada, Oklahoma |
| EPA/Athens        | U.S. Environmental Protection Agency, Ecosystems Research Division,<br>National Exposure Research Laboratory, Athens, Georgia         |
| EPA               | U.S. Environmental Protection Agency  |
| EPABAFEIII        | Bioavailable ferric iron measured by EPA/Ada  |
| ESTCP             | Environmental Security Technology Certification Program   |
| Fe II             | Ferrous iron  |
| Fe III            | Ferric iron   |
| FeRB              | Iron-reducing bacteria  |
| FRTR              | Federal Remediation Technologies Roundtable   |
| F <sub>s</sub>    | Soil fraction   |
| g/kg              | Grams per kilogram  |
| g/L               | Grams per liter   |
| GIT               | Georgia Institute of Technology   |
| HRC <sup>TM</sup> | Hydrogen Release Compound <sup>TM</sup>   |
| lb                | Pound   |
| MFEBRY            | Microcosm reducible ferric iron with BrY  |
| MFEBRYFeOOH       | Microcosm reducible ferric iron with BrY/FeOOH  |

|                 |   |
|-----------------|---|
| mg/kg           | Milligrams per kilogram                                       |
| MNA             | Monitored natural attenuation                                 |
| msl             | Mean sea level  |
| MTBE            | Methyl tertiary butyl ether                                   |
| N               | Number of samples   |
| NAS             | Naval Air Station   |
| NAVFAC          | Naval Facilities Engineering Command                          |
| NFESC           | Naval Facilities Engineering Services Center                  |
| NHD             | New Horizons Diagnostics Corporation                          |
| OU              | Operable Unit   |
| PCA             | Principal components analysis                                 |
| pH              | pH – negative log of hydrogen ion concentration               |
| PWIA            | Public Works Industrial Area                                  |
| RABITT          | Reductive Anaerobic <i>In Situ</i> Treatment Technology       |
| RMMAg           | Electron microprobe analysis, relative mass percent magnetite |
| RPD             | Relative percent difference                                   |
| SBIR            | Small Business Innovative Research                            |
| SCEC            | Support Center, Elizabeth City                                |
| SPLP            | Synthetic precipitation leaching procedure                    |
| SUBASE          | Submarine Base  |
| SVOC            | Semivolatile organic compound                                 |
| SW              | Solid waste   |
| T <sub>0</sub>  | Time 0 days   |
| T <sub>30</sub> | Time 30 days  |
| TCE             | Trichloroethene   |
| TCLP            | Toxicity characteristic leaching procedure                    |
| TEAPs           | Terminal electron accepting processes                         |
| TFE             | Total iron  |
| UC              | University of Colorado  |
| USAF            | U.S. Air Force  |
| USCS            | Unified Soil Classification System                            |
| USGS            | U.S. Geological Survey  |
| UST             | Underground storage tank                                      |
| V05FEII         | 0.5N HCl extractable ferrous iron                             |
| V05FEIII        | 0.5N HCl extractable ferric iron                              |
| V05FETOT        | 0.5N HCl extractable total iron                               |
| V6FEII          | 6N HCl extractable ferrous iron                               |
| V6FEIII         | 6N HCl extractable ferric iron                                |
| V6FETOT         | 6N HCl extractable total iron                                 |
| VC              | Vinyl chloride  |
| VOC             | Volatile organic compound                                     |
| ZHE             | Zero headspace extraction                                     |



## Preface

This report describes the demonstration and validation of a novel analytical technology: a bioavailable ferric iron (BAFeIII) assay. Demonstration and validation of the BAFelIII assay was conducted at four Department of Defense (DoD) installations.

CDM in cooperation with the Naval Facilities Engineering Services Center (NFESC) was the principal investigator. Several organizations assisted in the validation of the BAFelIII assay, including the U.S. Environmental Protection Agency (EPA), U.S. Geological Survey (USGS), Georgia Institute of Technology (GIT), and University of Colorado (UC). Individuals contributing to completion of this project are listed below:

|                              |            |
|------------------------------|------------|
| Carmen Lebron (PI)           | NFESC      |
| Barbara Sugiyama             | NFESC      |
| Patrick Evans, Ph.D. (Co-PI) | CDM        |
| Mary Trute                   | CDM        |
| Roger Olsen, Ph.D.           | CDM        |
| Rick Chappell, Ph.D.         | CDM        |
| John Wilson, Ph.D.           | EPA/Ada    |
| Cherri Adair                 | EPA/Ada    |
| Eric Weber, Ph.D.            | EPA/Athens |
| John Kenneke, Ph.D.          | EPA/Athens |
| B.T. Thomas, Ph.D.           | EPA/Athens |
| Tom DiChristina, Ph.D.       | GIT        |
| John Drexler, Ph.D.          | UC         |

This work also would not have been possible without the access to and help from the following DoD installations:

SUBASE Bangor, Washington  
Ft. Lewis, Washington  
NAS Pensacola, Florida  
US Coast Guard Support Center, Elizabeth City, North Carolina

Points of contact for this project are provided in Section 8.

## 1.0 Executive Summary

### 1.1 Background

A bioavailable ferric iron (BAFeIII) assay was invented and developed by CDM with funding from the U.S. Air Force. This assay is a standardized bioassay that directly measures the concentration of BAFeIII in soil or sediment. A BAFeIII test kit based on the assay is manufactured by New Horizons Diagnostics Corporation (NHD) of Columbia, Maryland.

BAFeIII is defined as follows:

*Ferric iron (Fe III) that is capable of being reduced by microorganisms that oxidize another chemical species and derive energy from the electron transfer.*

BAFeIII is an important terminal electron acceptor with significant assimilative capacity in many natural environments. Dissolved ferrous iron (Fe II) in groundwater is typically measured to assess Fe III reduction and calculate assimilative capacity, but this measurement underestimates this terminal electron accepting process because most Fe II remains bound to the soil. Dissolved Fe II also gives no indication of the amount of Fe III present in aquifer soil that is bioavailable. BAFeIII in the soil must be measured in order to quantify the true assimilative capacity of an aquifer.

Iron-reducing bacteria (FeRB) use and are dependent on BAFeIII. FeRB are known to oxidize or mineralize various organic compounds, such as benzene, toluene, vinyl chloride (VC), and methyl tertiary butyl ether (MTBE). Continued activity over a period of years is dependent on the presence of sufficient BAFeIII.

BAFeIII can also affect reductive dechlorination in MNA and EAB applications. BAFeIII can result in TCE being reductively dechlorinated to cDCE only and further reductive dechlorination can be inhibited (AFCEE, 2004). Thus knowledge of the BAFeIII concentration can indicate the potential for incomplete reductive dechlorination of TCE. It can also be used for planning EAB remedies. If the BAFeIII concentration is sufficient to inhibit cDCE reductive dechlorination, reductive dechlorination of TCE to cDCE and VC followed by oxidative biodegradation of VC and possibly cDCE under iron-reducing conditions may be a better approach.

### 1.2 Objectives of the Demonstration

The overall objective of this project was to demonstrate and validate the performance of the BAFeIII assay as an analytical technology for use in supporting bioremediation. Specific objectives were to:

- Validate the BAFeIII assay method using a combination of confirmatory analyses conducted by the U.S. EPA (EPA/Ada and EPA/Athens), Georgia Institute of Technology, and University of Colorado.
- Quantify costs associated with the technology.

### 1.3 Regulatory Drivers

Analysis of BAF<sub>Fe</sub>III is not required at this time and is considered optional by regulatory agencies. Additionally, no method for BAF<sub>Fe</sub>III has been approved by the EPA since it does not approve methods for unregulated compounds. The analyte (BAF<sub>Fe</sub>III) of interest in this demonstration is discussed in the EPA technical guidance on monitored natural attenuation (MNA) and enhanced anaerobic bioremediation (EAB) of chlorinated solvents (U.S. EPA, 1998; AFCEE, 2004). These documents review the use of BAF<sub>Fe</sub>III data to assess MNA of organic contaminants such as VC and consumption of injected electron donors during EAB.

### 1.4 Demonstration Results

**Table 1-1** presents validation results and indicates that the BAF<sub>Fe</sub>III assay is a precise analytical method for direct BAF<sub>Fe</sub>III quantification.

**Table 1-1. Performance Objectives and Results for the BAF<sub>Fe</sub>III Assay**

| Type of Performance Objective | Primary Performance Criteria  | Expected Performance (Metric) | Actual Performance Objective Met? |
|-------------------------------|---|-------------------------------|-----------------------------------|
| Qualitative                   | Relationship between BAF <sub>Fe</sub> III assay and degree of iron oxide crystallinity/surface area.           | Positive association          | Yes                               |
|                               | Relationship between BAF <sub>Fe</sub> III assay and confirmatory analyses.                                     | Positive association          | Yes                               |
|                               | Range of BAF <sub>Fe</sub> III assay relative to other analytical techniques.                                   | Similar or better range       | Yes                               |
|                               | Sample throughput of BAF <sub>Fe</sub> III assay.   | Labor time ≤ similar methods  | Yes                               |
|                               | Versatility of BAF <sub>Fe</sub> III assay.   | Consistent performance        | Yes                               |
| Quantitative                  | Intra-laboratory precision of BAF <sub>Fe</sub> III assay based on soil and laboratory replicates.              | Absolute RPD ≤ 35             | Yes                               |
|                               | Inter-laboratory precision of BAF <sub>Fe</sub> III assay based on replicates analyzed by both CDM and EPA/Ada. | -35 ≥ RPD ≤ 35                | Yes                               |

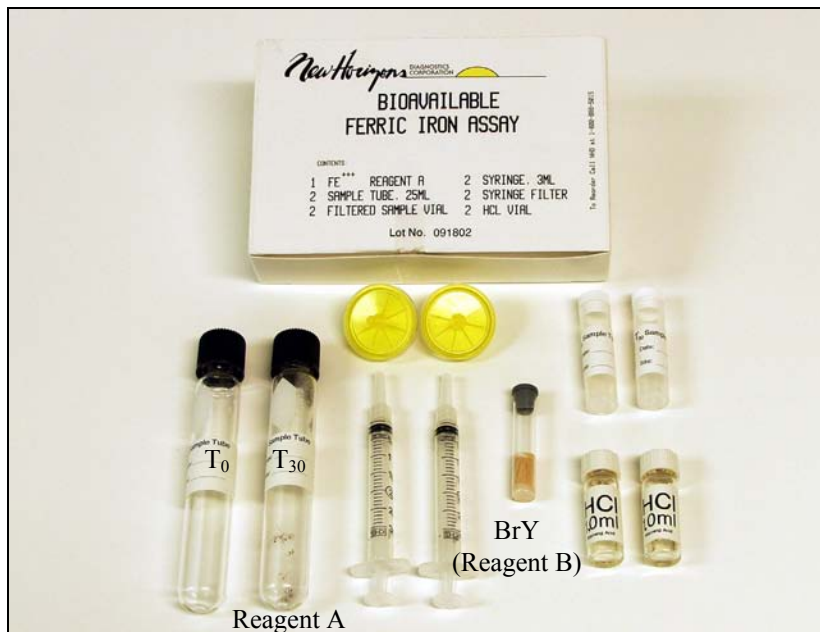
### 1.5 Stakeholder/End-User Issues

The BAF<sub>Fe</sub>III assay is an important tool that allows remedial project managers to obtain a more accurate and complete picture of site geochemistry and microbiology. This tool is useful in bioremediation projects involving natural attenuation and enhanced anaerobic bioremediation. Use of the direct BAF<sub>Fe</sub>III assay is recommended as a replacement for indirect chemical extraction methods. Additionally, it is recommended that BAF<sub>Fe</sub>III analysis of soil be conducted in addition to ferrous iron analysis in groundwater. The BAF<sub>Fe</sub> III assay purchase cost ranges from \$50 to \$75 each depending on the quantity purchased. Additional equipment, supplies, and labor are required and the estimated analysis cost was calculated to be \$212 each based on analysis of 6 samples. BAF<sub>Fe</sub>III analysis conducted by a commercial laboratory has been quoted at \$250 per analysis.

## 2.0 Technology Description

### 2.1 Technology Development and Application

The BAF<sub>Fe</sub>III test kit is pictured in **Figure 2-1**. The test kit is manufactured by New Horizons Diagnostics Corporation (NHD) of Columbia, Maryland. The BAF<sub>Fe</sub>III assay involves addition of a soil sample to a test tube that contains the lyophilized iron-reducing bacterium *Shewanella alga* BrY, lactate as an electron donor, and a mineral salts medium supplemented with reagents that accelerate the assay.



**Figure 2-1. BAF<sub>Fe</sub>III Assay Kit**

The BAF<sub>Fe</sub>III assay can be used for site characterization and monitoring in MNA and EAB applications. Natural attenuation of benzene-toluene-ethylbenzene-xylenes (BTEX) is one common example. Initial site characterization for MNA involves the calculation of assimilative capacity of an aquifer for biodegradation of BTEX. The BAF<sub>Fe</sub>III assay can be used to estimate the assimilative capacity in the aquifer material for BTEX biodegradation. These results can be used to determine the mass of BTEX that has been degraded previously and the potential for future BTEX biodegradation.

BAF<sub>Fe</sub>III can also affect reductive dechlorination in MNA and EAB applications. Reductive dechlorination is based on chlorinated compounds such as trichloroethene (TCE) serving as a terminal electron acceptor. Complete dechlorination of TCE to ethene requires that each dechlorination product (i.e., *cis*-dichloroethene [cDCE] and vinyl chloride [VC]) also serve as terminal electron acceptors. Terminal electron acceptors will be used preferentially according to thermodynamic and kinetic considerations. For example, VC may be dechlorinated to ethene

under methanogenic conditions (and correct microbial populations) but not under aerobic or denitrifying conditions in part because the free energies for reduction of oxygen and nitrate are greater (i.e., more negative) than for reduction of VC. The free energy for reduction of several BAF<sub>III</sub> oxides is greater than that for reductive dechlorination of cDCE to VC (Evans and Koenigsberg, 2001). BAF<sub>III</sub> can result in TCE being reductively dechlorinated to cDCE only and further reductive dechlorination can be inhibited (AFCEE, 2004). Thus knowledge of the BAF<sub>III</sub> concentration can indicate the potential for incomplete reductive dechlorination of TCE. It can also be used for planning EAB remedies. If the BAF<sub>III</sub> concentration is sufficient to inhibit cDCE reductive dechlorination, reductive dechlorination of TCE to cDCE and VC followed by oxidative biodegradation of VC and possibly cDCE under iron-reducing conditions may be a better approach.

A challenge in applying BAF<sub>III</sub> results is that insufficient experience exists to use the results in quantitative models at this time. Nevertheless, the results from the assay can be used in either a quantitative or qualitative manner. The BTEX example above represents a quantitative application of BAF<sub>III</sub> assay results. The enhanced anaerobic bioremediation application represents a qualitative application of the assay. Experience using the assay results in a qualitative fashion will lead to more quantitative applications as a database is developed. An example of a potential application is incorporation of BAF<sub>III</sub> as a variable in biodegradation computer modeling programs such as the EPA program BIOPLUME IV which is currently being beta-tested (John Wilson, personal communication). BIOPLUME is a two-dimensional, finite difference model for simulating the natural attenuation of organic contaminants in groundwater due to the processes of advection, dispersion, sorption, and biodegradation. The BIOPLUME program uses an USGS solute transport code and kinetic equations to determine the fate and transport of the organic contaminants and the electron acceptors (dissolve oxygen, nitrate, BAF<sub>III</sub>, sulfate, and carbon dioxide) and the reaction by-products (including dissolved Fe II). BioRedox-MT3DMS is another numerical fate and transport model that includes BAF<sub>III</sub> as an input parameter (Thompson et al. 2004).

## 2.2 Process Description

The procedure for the BAF<sub>III</sub> assay is graphically illustrated in **Figure 2-2** and includes the following steps following homogenization:

- Two 5-gram samples are placed into each of two 25-mL assay tubes labeled T<sub>0</sub> and T<sub>30</sub>.
- The T<sub>0</sub> tube, which is used to determine the initial or ambient concentration of Fe II present in the soil immediately following sample collection, contains no reagents or BrY, is filled with distilled water and 1 mL concentrated HCl, capped, then placed on a tube rotator for 48 hours, during which time weakly associated Fe II is extracted from the soil.
- Following the extraction period, the T<sub>0</sub> extract liquid is filtered, if necessary, and analyzed for initial Fe II.
- The T<sub>30</sub> tube is filled with distilled water plus the assay reagents, capped, mixed by hand, and then incubated in the dark at room temperature for 30 days. During the incubation period the iron-reducing bacteria (i.e., BrY) consume lactate and reduce BAF<sub>III</sub> to Fe II.

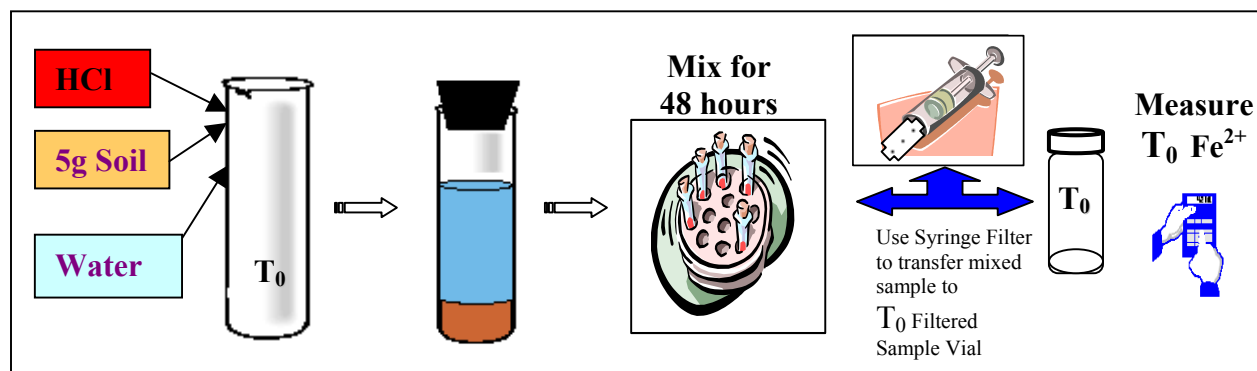
- Following the incubation period, 1 mL of liquid is withdrawn from the T<sub>30</sub> tube, discarded, replaced with 1 mL concentrated HCl to create a 0.5N HCl solution, then the tube is placed on a tube rotator for 48 hours, during which time both initial Fe II and Fe II produced by BAF<sub>Fe</sub> III reduction are solubilized.
- Following the extraction period, the T<sub>30</sub> liquid is filtered and analyzed for Fe II.
- The concentration of Fe II in the T<sub>30</sub> extract liquid is the total Fe II – the sum of ambient Fe II (T<sub>0</sub> tube) and BAF<sub>Fe</sub> III. The following formula is used to calculate BAF<sub>Fe</sub> III:

$$\text{BAFeIII}(\text{mg/kg}) = \frac{(C_E \text{ in } T_{30}) - (C_E \text{ in } T_0)}{217 F_S}$$

C<sub>E</sub> is the measured concentration of Fe II in the extract liquid (mg/L) and F<sub>S</sub> is the solids fraction (g dry soil/g wet soil).

- Extract Fe II concentrations (C<sub>E</sub>) are measured using a Hach test kit (Hach Company, Method 8146) followed by dilution. Dilution requirements are determined using Quantofix® Iron 1000 test strips (VWR Part No. 60787-724) without the Iron 1 reagent.

## STEP 1:



## STEP 2:

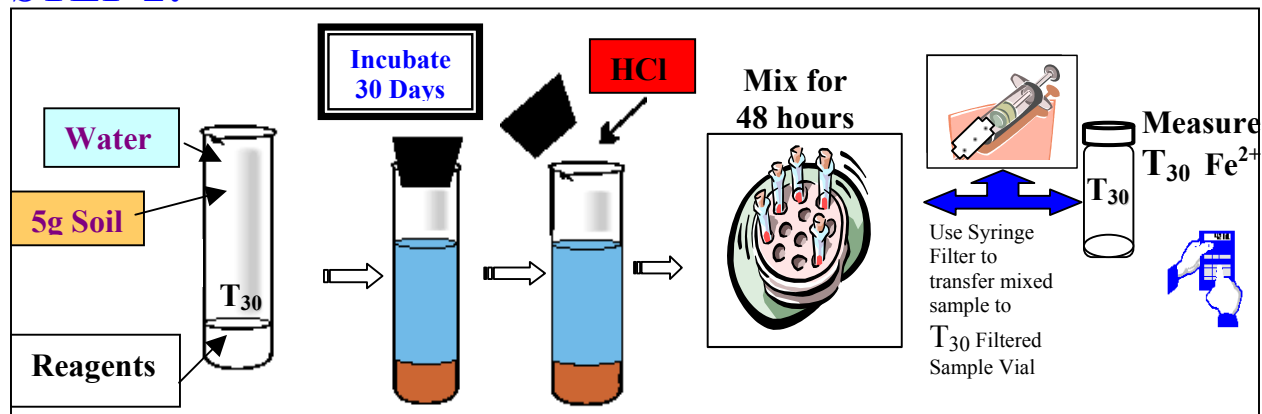


Figure 2-2. BAF<sub>Fe</sub> III Assay Kit Procedure

## 2.3 Previous Testing of the Technology

Initial development and preliminary field-testing of the BAF<sub>Fe</sub>III assay technology was conducted under a Phase I Small Business Innovative Research (SBIR) grant from the USAF (Evans, 1997; Evans, 2000). Further development and field-testing of the technology was conducted under Phase II of the SBIR, which led to development of a field test kit (Evans and Jones, 1999; Evans et al., 1999).

## 2.4 Advantages and Limitations of the Technology

Advantages and limitations of the BAF<sub>Fe</sub>III assay are summarized in **Table 2-1**. These advantages are described in detail below.

**Table 2-1. Advantages and Limitations of the BAF<sub>Fe</sub>III Assay**

| Category                      | Advantages  | Limitations  |
|-------------------------------|---|--|
| <b>Analytical Methodology</b> | Direct method that uses a bioassay rather than an indirect chemical extraction.     | Bacteria must be stored frozen prior to use.   |
|                               | Facultative bacterium is used that does not need to be stored anaerobically.        | Uses <i>Shewanella alga</i> BrY which may not be representative of all sites.                                |
|                               | Assay composition and method has been standardized.                                 | 30-day incubation time.  |
| <b>Sampling Requirements</b>  | Frequent sampling is not necessary.   | Requires soil or sediment samples and invasive sampling for their procurement.                               |
|                               | Requires 5 grams of soil per analysis.  |  |
| <b>Technology Application</b> | Indicates BAF <sub>Fe</sub> III electron donor demand for MNA and EAB applications. | No reference method for BAF <sub>Fe</sub> III analysis exists.   |
|                               | Analysis is more robust than commonly used chemical extraction methods.             | Can give maximum values for BAF <sub>Fe</sub> III because of presence of electron shuttles in assay reagent. |

BAF<sub>Fe</sub>III data allow site managers and regulators to evaluate MNA and EAB at sites more completely and accurately than with dissolved Fe II data alone. In the case of BTEX natural attenuation, dissolved Fe II data allow calculation of the mass of BTEX that has been biodegraded historically and is being biodegraded currently. BAF<sub>Fe</sub>III data allow calculation of the mass of BTEX that will be biodegraded in the future. It is impossible to calculate future potential for BTEX biodegradation using dissolved Fe II data alone. Furthermore, since most dissolved Fe II remains bound to the soil, the historical and current mass of biodegraded BTEX is underestimated using dissolved Fe II data for electron acceptor calculations. Completion of a mass balance and subsequent understanding of contaminant source fate is dependent on accurate electron acceptor calculations. In the case of EAB of TCE, BAF<sub>Fe</sub>III data allow determination of the total electron donor demand. High electron donor demand can decrease the likelihood that TCE will be reductively dechlorinated beyond cDCE to VC and ethene upon addition of an electron donor such as molasses, lactic acid, vegetable oil, or HRC<sup>TM</sup> (Evans and Koenigsberg, 2001; AFCEE, 2004). High electron donor demand can also prevent complete reductive dechlorination under MNA conditions. Dissolved Fe II data alone give no indication of this electron donor demand.

While the BAF<sub>FeIII</sub> assay provides these advantages over measurement of dissolved Fe<sup>II</sup>, it does depend on soil sampling in the saturated zone, which is costly and inconvenient for routine sampling. On the other hand, measurement of BAF<sub>FeIII</sub> in soil likely does not require quarterly sampling of numerous locations. This decreased sampling frequency can minimize the additional cost associated with soil sample collection.

The BAF<sub>FeIII</sub> assay evaluated in this report is in a sense a standardized bioassay. Besides being the first of its kind, the assay has many advantages that make it an easy-to-use and reliable analytical tool. Unlike laboratory-based microcosm studies, it is standardized, self-contained, portable, packaged for field or laboratory use, and includes lyophilized FeRB that are relatively stable. Care must be taken to store the lyophilized FeRB under freezing conditions for stability. The bioassay reagents other than FeRB are packaged separately from the FeRB and are stable at room temperature. These chemical components are present at optimal levels and are known to influence bioavailability. Their presence is intended to provide reproducible, standardized, and direct estimates of the maximum concentration of BAF<sub>FeIII</sub> in a given soil sample. Recognition that the assay results are maximum values should be considered when using the data. For example, the amount of electron donor required to overcome iron reduction alone in an enhanced anaerobic bioremediation scenario may be less than predicted based on BAF<sub>FeIII</sub> assay results.

A potential limitation of the BAF<sub>FeIII</sub> assay is that the indigenous FeRB may be different in their iron-reducing capabilities when compared to the strain used in the assay (i.e., *Shewanella alga* BrY). Inclusion of BrY in the assay was intended to make the assay standardized and reproducible. Additionally, since BrY is a facultative microorganism, storage under anaerobic conditions is not necessary, further increasing the test kit's ease-of-use. BAF<sub>FeIII</sub> is an operationally defined analyte (i.e., the measured value of the analyte is dependent on the method used for its analysis) and use of BrY is part of this operational definition. The BrY-based assay yields a reproducible maximum value of BAF<sub>FeIII</sub> in a given sample. The decision whether or not to use BrY in the bioassay represents a trade-off of obtaining site-specific results versus standardization, reproducibility, and ease-of-use. If results using only indigenous bacteria are desired, the BrY culture can easily be left out of the assay since it is packaged separately. Iron reduction would then be accomplished via FeRB that are indigenous to the soil sample used in the assay. A new limitation would be introduced by conducting the assay in this manner, however, since the required incubation time would be unknown. Monitoring of the assay over time would be required which would decrease the ease-of-use of the assay. Direct comparison of BAF<sub>FeIII</sub> results to results for other sites would also not be possible. In addition to BrY, electron shuttles (i.e., humic acids and AQDS) are included in the assay. The inclusion of electron shuttles is also part of the operational definition. Their inclusion increases the reliability and speed of the assay and also can result in determination of maximum BAF<sub>FeIII</sub> values.

Another potential limitation of the BAF<sub>FeIII</sub> assay involves the one-month incubation time. However, considering that standard turnaround time for most analytical laboratories is two weeks, this time requirement is acceptable in most cases.



Finally, no standardized technologies exist for directly measuring BAF<sub>FeIII</sub>. Other methods that have been used or evaluated for BAF<sub>FeIII</sub> measurement are presented in **Table 2-2** and discussed below.

**Table 2-2. Other Methods for BAF<sub>FeIII</sub> Measurement**

| <b>Category</b>                      | <b>Method</b>  | <b>Advantages</b>  | <b>Limitations</b>  |
|--------------------------------------|--|--|---|
| <b>Chemical Extraction</b>           | <ul style="list-style-type: none"> <li>• 0.5 N HCl</li> <li>• 6 N HCl</li> <li>• Hydroxylamine HCl</li> <li>• Citrate dithionite bicarbonate</li> <li>• Ammonium oxalate</li> </ul>                              | <ul style="list-style-type: none"> <li>• Easy to use</li> <li>• Inexpensive</li> </ul>   | <ul style="list-style-type: none"> <li>• Indirect</li> <li>• Indicates chemical extractability rather than bioavailability</li> <li>• Does not accurately represent true bioavailability of different crystalline phases</li> </ul> |
| <b>Redox Titration</b>               | <ul style="list-style-type: none"> <li>• AHDS titration</li> </ul>   | <ul style="list-style-type: none"> <li>• Data indicate good correlation with microcosms</li> </ul>                               | <ul style="list-style-type: none"> <li>• Not commercially available</li> <li>• Requires anaerobic conditions</li> </ul>   |
| <b>Sophisticated Instrumentation</b> | <ul style="list-style-type: none"> <li>• Electron microscopy</li> <li>• Electron microprobe</li> <li>• X-ray diffraction</li> <li>• Near infrared spectrophotometry</li> <li>• Mössbauer spectroscopy</li> </ul> | <ul style="list-style-type: none"> <li>• Potential identification of specific crystalline phases</li> </ul>                      | <ul style="list-style-type: none"> <li>• Expensive</li> <li>• Some methods are insufficiently sensitive</li> </ul>  |
| <b>Treatability Study</b>            | <ul style="list-style-type: none"> <li>• Microcosm</li> </ul>  | <ul style="list-style-type: none"> <li>• Potentially the best simulation of actual site geochemistry and microbiology</li> </ul> | <ul style="list-style-type: none"> <li>• Expensive</li> <li>• Not standardized</li> </ul>   |

Chemical extraction, sophisticated instrument-dependent methods, and microcosm studies have been evaluated, but each has significant disadvantages. Selective extraction using a variety of extractants, including various concentrations of HCl, hydroxylamine-HCl, ammonium oxalate, citrate, citrate dithionite bicarbonate and other compounds has been used to attempt to quantify BAF<sub>FeIII</sub>. However, these extractants do not provide direct measurements and do not necessarily correlate to the concentration of BAF<sub>FeIII</sub> (Lovley and Phillips, 1987). Also, extraction methods do not take into account the effect of groundwater chemistry on bioavailability. A laboratory method for BAF<sub>FeIII</sub> quantitation involving redox titration of soil with the reduced form of anthraquinone disulfonate (AQDS) also known as anthraquinol disulfonate (AHDS) has been evaluated (Hacherl et al., 2001). This method is not readily available. Sophisticated instrumentation, including electron microscopy, electron microprobe analysis, near infrared spectrophotometry, and Mössbauer spectroscopy have been evaluated but are not especially useful. Furthermore, these techniques are expensive and not readily available. Microcosm studies have been conducted in various laboratories but with different methods and media.

While microcosm studies are a direct approach to evaluation of BAF<sub>FeIII</sub>, no standard method exists for conducting them; they are also time-consuming and expensive. Therefore, the major advantage of the BAF<sub>FeIII</sub> assay over other methods is that it is a standardized and direct measurement of BAF<sub>FeIII</sub>.

### 3.0 Demonstration Design

#### 3.1 Performance Objectives

The BAF<sub>FeIII</sub> assay is difficult to validate because no standard method exists to measure bioavailability of ferric iron. Nevertheless, performance criteria were developed *a priori* in order to be able to validate the BAF<sub>FeIII</sub> assay. These criteria were based initially on the demonstrated relationship between Fe III bioavailability and Fe III oxide particle surface area (Roden and Zachara, 1996). Different Fe III oxides ranging from amorphous ferric oxyhydroxide to various crystalline forms have different specific surface areas. Oxides with greater specific surface area (amorphous oxides having the greatest) have been shown to be more bioavailable for iron reduction (Roden and Zachara, 1996). Thus the initial working hypothesis of the evaluation was that the BAF<sub>FeIII</sub> concentration determined by the assay should correlate to the specific surface area of the oxide particles in a soil sample. In addition, other factors associated with groundwater may influence Fe III bioavailability (Evans, 2000; Roden and Urrutia, 2002) including pH, specific conductivity, divalent cations, electron shuttles such as humic acids, chelators, and adsorbed anions including ferrous iron. Performance objectives for the demonstration were based in part on these multiple factors and are presented in **Table 3-1**.

**Table 3-1: Performance Objectives for BAF<sub>FeIII</sub> Assay**

| Type of Performance Objective | Primary Performance Criteria   | Expected Performance (Metric) | Actual Performance Objective Met? |
|-------------------------------|--|-------------------------------|-----------------------------------|
| <b>Qualitative</b>            | Relationship between BAF <sub>FeIII</sub> assay and degree of iron oxide crystallinity/surface area.           | Positive association          | Yes                               |
|                               | Relationship between BAF <sub>FeIII</sub> assay and confirmatory analyses.                                     | Positive association          | Yes                               |
|                               | Range of BAF <sub>FeIII</sub> assay relative to other analytical techniques.                                   | Similar or better range       | Yes                               |
|                               | Sample throughput of BAF <sub>FeIII</sub> assay.   | Labor time ≤ similar methods  | Yes                               |
|                               | Versatility of BAF <sub>FeIII</sub> assay.   | Consistent performance        | Yes                               |
| <b>Quantitative</b>           | Intra-laboratory precision of BAF <sub>FeIII</sub> assay based on soil and laboratory replicates.              | Absolute RPD ≤ 35             | Yes                               |
|                               | Inter-laboratory precision of BAF <sub>FeIII</sub> assay based on replicates analyzed by both CDM and EPA/Ada. | -35 ≥ RPD ≤ 35                | Yes                               |

#### 3.2 Selection of Test Sites

Selection of sites was based on the following criteria:

- Availability of an existing groundwater monitoring well network.
- Geological and hydrogeological characteristics.

- Terminal electron accepting processes (TEAPs) occurring in the aquifer.
- Concentrations of parent compounds and presence of daughter products.
- Groundwater chemistry.
- Ability to drill on site.
- Availability and quality of existing site characterization documentation.

The objective was to select sites that offered a range of iron concentrations, geochemical characteristics, and terminal electron accepting processes, to enable validation of the BAF<sub>FeIII</sub> assay. Four test sites were used for the demonstration of the BAF<sub>FeIII</sub> assay:

- Bangor Naval Submarine Base in Kitsap County, Washington (SUBASE Bangor) – dissolved petroleum hydrocarbons and chlorinated VOCs.
- Fort Lewis Logistics Center near Tillicum, Washington (Fort Lewis) – chlorinated VOCs.
- Naval Air Station (NAS) in Pensacola, Florida (NAS Pensacola) – chlorobenzene and TCE.
- U.S. Coast Guard Support Center in Elizabeth City, North Carolina – Fuel Farm site with petroleum hydrocarbons and MTBE and North Beach site with chlorinated hydrocarbons.

### **3.3 Test Site History/Characteristics**

Summaries of the four demonstration site are provided in this section. Additional details are available in the Technology Demonstration Plan (CDM, 2001) and the Final Report (NAVFAC, 2005).

#### ***SUBASE Bangor***

The study area for this demonstration is the vicinity of Operable Unit 8 (OU8), located in the Public Works Industrial Area (PWIA) of SUBASE Bangor. SUBASE Bangor is located near the town of Silverdale, Washington. An onsite UST is believed to be the source of a release of unleaded gasoline into the surrounding media between 1982 and 1986. In 1986, soil vapor extraction/air system and product recovery were implemented to clean up the site. To date, liquid petroleum hydrocarbons remain in several monitoring wells at the PWIA (EA, 2000). Chlorinated VOCs are also present in site groundwater (EA, 2000).

Geological conditions at OU8 at SUBASE Bangor have been highly characterized by drilling and monitoring well installation. The area consists of four stratigraphic units: construction fill, Vashon till (Qvt), Vashon Advance Outwash (Qva), and Lawton Clay. The construction fill can be found 2 to 3 feet bgs and consists of a sandy material. Underlying the construction fill and ranging to a depth of about 45 ft bgs is the Vashon till, which consists of silt, sand, gravel, and cobbles. This unit is 20 to 40 ft thick. The Vashon Advance Outwash (location of the shallow aquifer) is beneath the Vashon till and consists of sand, silt, and gravel. The thickness of the Vashon Advance Outwash is about 100 to 130 feet. Beneath the Vashon Advance Outwash is

the Lawton Clay aquitard. A silty transition zone in the bottom of the Vashon Advance Outwash separates the shallow aquifer from the lower aquitard.

### ***Fort Lewis***

The study area for this demonstration is the vicinity of the East Gate Disposal Yard (EGDY) of the Fort Lewis Logistics Center (Fort Lewis), located south of Tacoma, Washington. The EG DY, which is situated at the northwest corner of the base, originally was used for storage and disposal of various solid and liquid waste products. Since 1982, studies have been conducted at the EG DY to verify and delineate contamination at the site. Affected media were soil and groundwater, with the prominent contaminant being trichloroethene (TCE) (Battelle, 2000).

The upper portion of the EG DY at Fort Lewis consists of a brown to black alluvial sand and gravel matrix with local lenses of silts. The material gets coarse with depth. Underlying this formation at about 260 feet msl is the Vashon Till, which is a complex mixture of silt, sand, and clay. The Vashon Till has low permeability and serves as a barrier between the upper and deeper aquifers. At the source area the groundwater can be encountered between 8 and 15 feet bgs. Farther downgradient the groundwater is generally between 10 and 35 feet bgs. The upper aquifer is unconfined and mostly anaerobic. Groundwater flow is generally west to northwest. There are more than 80 monitoring wells and piezometers on site.

Battelle Memorial Institute (in cooperation with the Air Force Research Laboratory, USGS, EPA, and Cornell University) performed Reductive Anaerobic *In Situ* Treatment Technology (RABITT) at the EG DY of Fort Lewis, and further site characterization details can be found in their report (Battelle, 2000). The BAF eIII demonstration was done in the vicinity of the RABITT demonstration.

### ***NAS Pensacola***

The study area for this demonstration is the vicinity of the wastewater treatment plant at the NAS in Pensacola, Florida (NAS Pensacola), located near Pensacola Bay in the far northwest corner of the state (USGS, 1999).

The area predominantly consists of marine and fluvial terrace deposits ranging from fine- to medium-grained sands, silts, clays, and gravel. The site has two aquifers, a shallow aquifer and a deeper confined aquifer (referred to as the underlying main producing zone). There is a 20-foot-thick confining barrier of low-permeable silts and clays that separate the upper and lower aquifers. The upper aquifer is composed of fine- to medium-grained sands. The main producing zone is used locally as a water supply and consists of permeable sands and gravel. Two plumes have been identified at the site, one comprised of chlorinated ethenes and the other chlorinated benzenes. Most of the contaminants on site are located in the upper aquifer region. The depth of contamination ranges from 20 to 40 feet bgs.

### ***Elizabeth City***

The U.S. Coast Guard Support Center in Elizabeth City, North Carolina, is located on the southern bank of the Pasquotank River. Two separate areas at the site were used in this demonstration, the fuel farm area (petroleum hydrocarbon) and the North Beach area (chlorinated VOCs).

The following description of the fuel farm area was obtained from the report by Wilson, et al. (2000). The former fuel farm was located south of a concrete ramp used to recover seaplanes from the Pasquotank River. A plume of MTBE and fuel hydrocarbons in ground water emanates from a source area in the location of the former fuel farm, and flows under the concrete ramp toward the Pasquotank River to the north, and toward a drainage canal along the western side of the seaplane ramp. This source area corresponds to the former location of fuel storage tanks on the site. Fuel was stored at the site until December 31, 1991. The fuel farm had been in use since 1942, and originally consisted of a 50,000-gallon concrete underground storage tank and two steel underground storage tanks with a volume of 12,000-gallons and 15,000-gallons, respectively. The steel tanks were apparently removed in the mid-1980s. In addition to the underground storage tanks, two steel, aboveground storage tanks with a capacity of 50,000 gallons were installed in the mid-1980s. There was evidence of corrosion in the transfer lines from these tanks. They were taken out of service and removed from the site. No evidence of a release from the pipes was discovered. The U.S. Coast Guard began a free product recovery effort at the site in September 1990. Eight recovery wells were arranged around the source area in a circle. By March 1992, a total of 79,000 gallons of fuel was recovered.

The following description of the North Beach Disposal Area was provided by ARCADIS (2004). The North Beach Disposal Area occupies 4.8 acres in the northeast corner of the Support Center, Elizabeth City (SCEC). The site is bounded immediately north and west by the Pasquotank River and to the east by a drainage canal. The North Beach site is unpaved. Approximately half of the site is heavily wooded. The other half, where the majority of disposal activities may have occurred, consists of grass-covered open areas. Historical information and site investigation activities indicate that industrial wastes generated at the SCEC may have been buried at the North Beach Disposal Area. The exact quantity and nature of the wastes disposed of in the North Beach Disposal Area are unknown; however, it is suspected that the wastes may have included chlorinated solvents, batteries, petroleum wastes, scrap metals, paint sludges, and plating wastes. Disposal activities likely occurred from the 1940s to approximately 1975. Four separate areas of concern (i.e., Source Areas 1, 2, 3, and 4) were identified at the site and had elevated concentrations of metals, scrap-metal fragments, VOCs, and semivolatile organic compounds (SVOCs) in soil. Only PCE, TCE, cis-1,2-DCE, vinyl chloride and pentachlorophenol are present in groundwater at elevated concentrations.

Hydrogen Release Compound™ (HRC™), a food-grade polylactate ester, was injected into the shallow aquifer zone at multiple points near Source Area 2 of the North Beach Disposal Area from January 21 to 25, 2003. The treatment area for HRC injection is a grid approximately 40 feet wide by 100 feet long, encompassing Monitor Wells GP20, GM315, GM330, and GM360. Within the grid area, Standard HRC was injected into 40 points while HRC Primer was injected

into 9 points. A total of 5,545 pounds of HRC was injected across the grid, with between 110 and 135 pounds of Standard HRC or primer injected at each point. The depths for these injections were 5 feet below ground surface (ft bgs) to 45 ft bls within the primary interval impacted by chlorinated VOCs. Quarterly monitoring has been conducted for one year since the HRC injections. Results indicate that HRC does not appear to have significant influence on groundwater geochemistry beyond the immediate vicinity of the injection points within the grid.

### **3.4 Physical Set-up and Operations**

On-site operations involved collection and packaging of samples as described in Section 3.5. Site visits were conducted as follows:

Site 1 – SUBASE Bangor: January 22 to February 2, 2001

Site 2 – Fort Lewis: February 19 to March 2, 2001

Site 3 – NAS Pensacola: April 29 to May 3, 2002

Site 4 – Elizabeth City: October 23 to 25, 2002

Physical operation and set-up of the BAF<sub>Fe</sub>III test kit was conducted as described in Section 2.2.

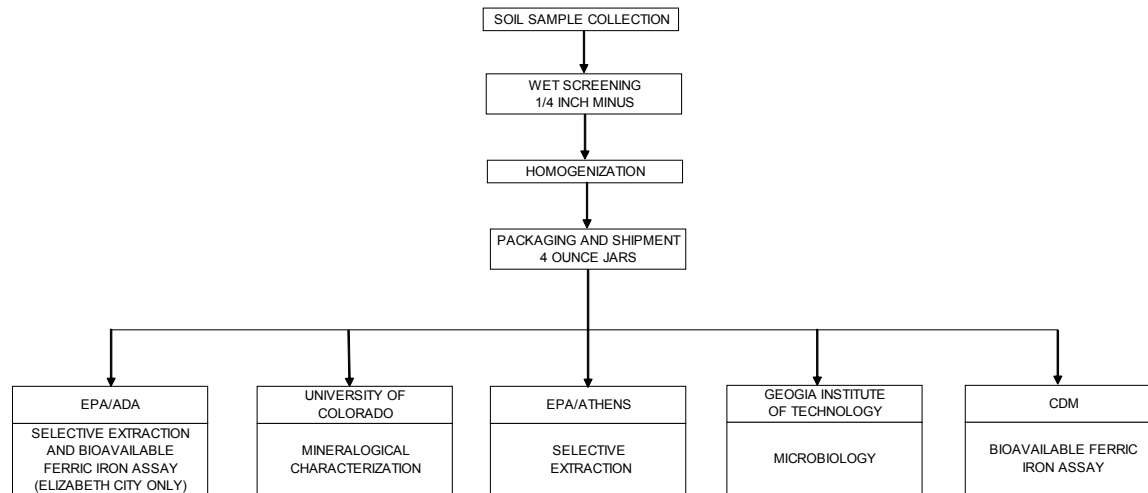
### **3.5 Sampling/Monitoring Procedures**

Groundwater samples were collected from existing monitoring wells on each site using low flow techniques and a peristaltic or bladder pump system. Soil borings were completed for collection of soil samples using hollow-stem auger, direct push technology, or hand-auger. During drilling, a CDM engineer or scientist logged and sampled the borings. The soils were visually described and classified in accordance with the Unified Soil Classification System (USCS; ASTM D2488-84). Generally two sections of each boring were collected (i.e., the top and bottom portions). Specific sample locations and depths are presented in the Final Report (NAVFAC, 2005). Attempts were made to obtain different types of soil samples as defined by USCS. The soil from each section was homogenized by hand (mixing with stainless steel spoon in a bowl) and then placed in 4- or 8-ounce glass jars, capped with Teflon<sup>TM</sup>-lined lids, and labeled prior to shipment to the labs. Samples were shipped in coolers with ice to maintain temperature between 2 and 6 °C. Soil samples were sent to the CDM laboratory in Bellevue, Washington for BAF<sub>Fe</sub>III analysis. Soil samples were also sent to other organizations for analysis as detailed in **Figure 3-1**.

### **3.6 Analytical Procedures**

**Figure 3-1** illustrates the different analyses that were conducted to validate the BAF<sub>Fe</sub>III assay. Appendices H and I of the Technology Demonstration Plan (CDM, 2001) and Appendix A of the Final Report (NAVFAC, 2005) include detailed analytical procedures that were conducted to demonstrate and validate the BAF<sub>Fe</sub>III assay. The significance of each analysis relative to BAF<sub>Fe</sub>III is provided, along with the method description and the organization that conducted the analysis.

Analyses were conducted immediately after sample collection with the following exceptions:  
1) BAF<sub>FeIII</sub> analysis of samples collected from SUBASE Bangor and Ft. Lewis were conducted on January 11, 2002 on archived samples, and 2) HCl extractions were repeated in March, 2002 using the ferrozine analysis method.



**Figure 3-1. Soil Sample Allocation and Analysis**

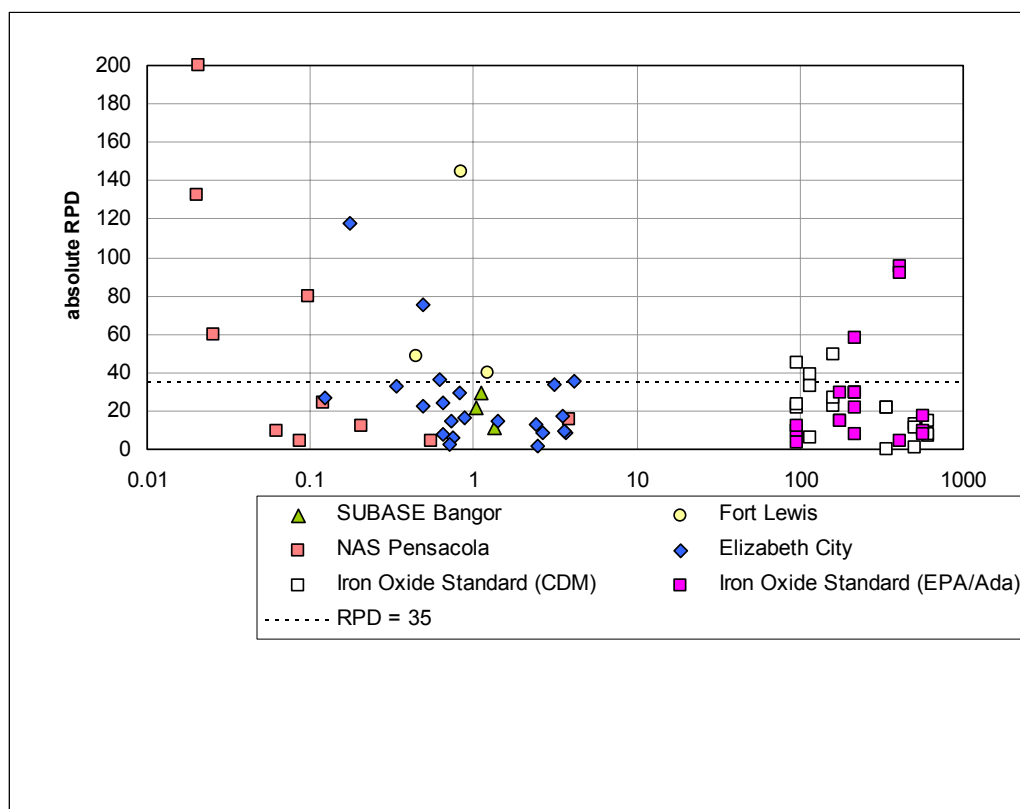


## 4.0 Performance Assessment

### 4.1 Performance Data

This section presents a brief summary of performance data for the BAF<sub>FeIII</sub> assay. A more complete description of these data is presented in the Final Report (NAVFAC, 2005).

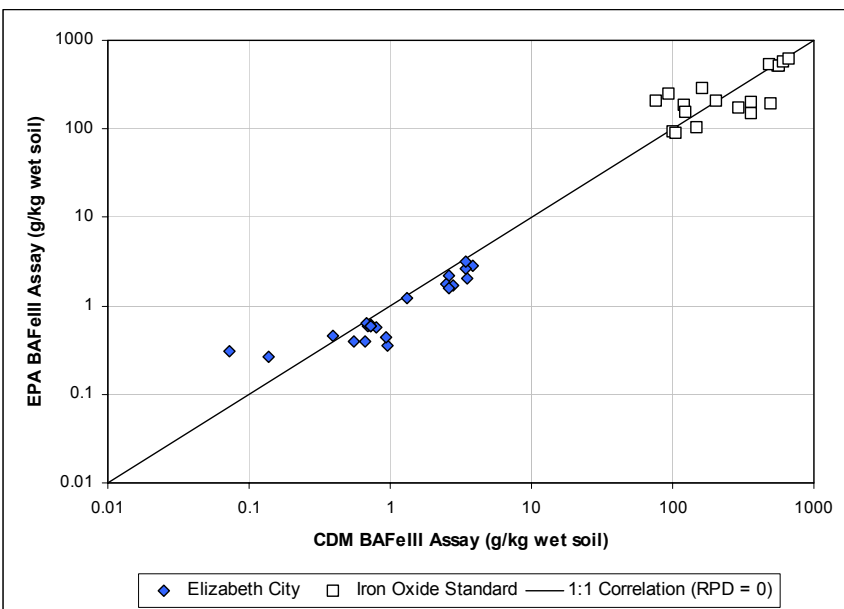
Replicate analyses were conducted to demonstrate the intra-laboratory precision of the BAF<sub>FeIII</sub> assay. An absolute RPD of 35 was used to evaluate the replicate data. This value was selected as an approximate criterion for analyses of replicates of inherently non-homogeneous soils. Further discussion of the valid use of an RPD of 35 can be found in EPA guidance on analysis of solid matrices (U.S. EPA, 2002). The overall average absolute RPD for the 76 CDM intra-laboratory replicates was 29.7, which met the  $RPD \leq 35$  criterion, with absolute RPDs for most of the individual replicates (77.6%) also meeting the criterion (**Figure 4-1**).



**Figure 4-1. Intra-Laboratory Replicate Precision for BAF<sub>FeIII</sub> Assay**

Replicate analyses were also conducted to demonstrate the inter-laboratory precision of the BAF<sub>FeIII</sub> assay. An RPD of 0 was used to evaluate the replicate data. This value was selected to represent no difference between the analyses as conducted by the two laboratories, i.e., a perfect 1:1 correlation and no inter-laboratory bias. The overall average RPD for the 40 inter-laboratory replicates was 12, which indicated that the CDM results were slightly higher, on average, than

the EPA results (**Figure 4-2**) but the difference was not statistically significant. The correlation coefficient for the log-transformed data was 0.98. For the aquifer samples, 25% of the samples agreed within a factor of 20%, 50% agreed within a factor of 37%, 75% agreed within a factor of 66%, and all of the samples agreed within a factor of 170%. For the iron oxide samples, 25% of the samples agreed within a factor of 8%, 50% agreed within a factor of 29%, 75% agreed within a factor of 63%, and all of the samples agreed within a factor of 160%.



**Figure 4-2. Inter-Laboratory Replicate Precision for BAFelIII Assay**

Principal components analysis (PCA) was conducted to evaluate the relationships and associations among the various potential bioavailable Fe III factors. PCA is a statistical method of identifying correlations of a large number of variables by grouping inter-related variables into “components”. Results indicated that approximately 43% of the total soil data set variance was explained by the first two components. The correlations between the original variables and the components are referred to as “loadings”. Thus, variables with high loadings in a particular component are associated with each other (i.e., they are intra-correlated). A listing of the variables with loadings greater than 0.45 in the first 2 components is provided in **Table 4-1** (see the list of acronyms for definitions).

**Table 4-1: Component Loadings**

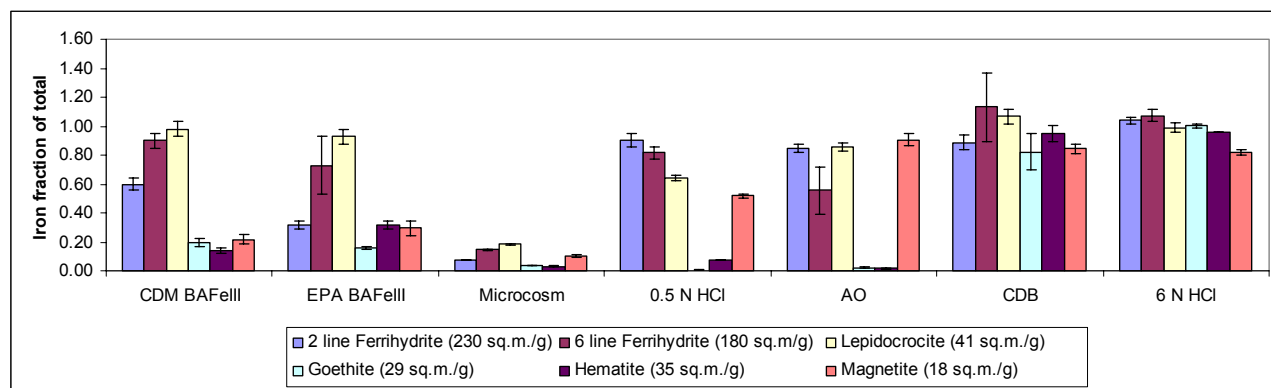
| Component | Variables with Loadings > 0.45   |
|-----------|--|
| 1         | V6FETOT, V6FEII, RMMAG, V6FEIII, V05FETOT, V05FEIII, MFEFBRY, V05FEII                        |
| 2         | V6FEIII, V05FETOT, V05FEIII, MFEFBRY, AOFE, EPABAFEIII, TFE, CDBFE, MFEFBRYFEOOH, CDMBAFEIII |

Component 1, which accounted for approximately 22% of the total variance in the data set, contained a number of factors that were associated with each other but not with the BAFelIII

assay. Component 1 was concluded to be associated with iron as opposed to BAF<sub>FeIII</sub>. Component 2, which accounted for approximately 20% of the total variance in the data set, contained several of the same variables that loaded highly into Component 1. Component 2 also contained the CDM and EPA BAF<sub>FeIII</sub> assay variables and the confirmatory analyses citrate dithionite bicarbonate and ammonium oxalate extractable Fe, total Fe, and iron oxide (FeOOH)-supplemented microcosm with BrY. Component 2 was concluded to be associated with BAF<sub>FeIII</sub>. These results demonstrated that positive associations exist between the BAF<sub>FeIII</sub> assay and confirmatory analyses that are related to and indicative of BAF<sub>FeIII</sub>.

**Figure 4-3** shows, for the synthetic iron oxides, iron concentrations measured using the BAF<sub>FeIII</sub> assay, microcosms, and chemical extractions, all being expressed as fractions of total iron concentrations. The results for the BAF<sub>FeIII</sub> assays conducted by CDM and EPA were qualitatively similar to results of microcosms conducted with *Shewanella alga* BrY. Results for the chemical extractions were qualitatively different from the BAF<sub>FeIII</sub> assay results. These data demonstrate the BAF<sub>FeIII</sub> assay yields a more representative estimate of iron oxide bioavailability than do chemical extractions.

CDM and EPA BAF<sub>FeIII</sub> assay results for 6-line ferrihydrite, lepidocrocite, and magnetite were not significantly different (*p* values ranged from 0.28 to 0.48). CDM and EPA BAF<sub>FeIII</sub> assay results for 2-line ferrihydrite and hematite were significantly different. The CDM value was 90% greater than the EPA value for 2-line ferrihydrite (*p* = 0.0074). The CDM value was 56% less than the EPA value for hematite (*p* = 0.011).



**Figure 4-3. Summary of Iron Oxide Analytical Results**

**Figure 4-3** also shows the specific surface area for each oxide. Goethite, hematite, and magnetite had relatively low BET surface areas and correspondingly low BAF<sub>FeIII</sub> fractions based on the BAF<sub>FeIII</sub> assay. Poorly crystalline, high surface area iron oxides such as 2-line and 6-line ferrihydrite demonstrated greater BAF<sub>FeIII</sub> fractions than highly crystalline, low surface area iron oxides such as magnetite, hematite, and goethite. Such a relationship between bioavailability and surface area has been demonstrated previously (Roden and Zachara, 1996). On the other hand, lepidocrocite had a relatively low surface area and yet the highest BAF<sub>FeIII</sub> fraction. Previous investigations have demonstrated that lepidocrocite has a high bioavailability

even though its surface area is low (Roden, 2003; Schwertmann et al., 1986). The relatively high bioavailability of lepidocrocite appears to be related to its crystal structure (Cooper et al., 2000; Hersman et al., 2001). These data further indicate that factors other than surface area affect iron oxide bioavailability. Thus the direct BAF<sub>III</sub> bioassay yields results that are more representative of biological iron oxide reduction than are indirect chemical extractions.

A mass balance calculation on iron at the Elizabeth City Fuel Farm site was conducted to further validate the BAF<sub>III</sub> assay and illustrate the use of BAF<sub>III</sub> data. The mass balance was conducted by calculating the mass of BAF<sub>III</sub> originally present in the area impacted by hydrocarbons, calculating the mass of ferrous iron removed in soluble form via downgradient groundwater transport, and then comparing the two values. Comparisons of the calculated iron masses are presented in **Table 4-2**. These results indicate that the BAF<sub>III</sub> assay did not underestimate the amount of BAF<sub>III</sub> iron present in the soil and thus gave a more robust BAF<sub>III</sub> estimate. Estimates of BAF<sub>III</sub> obtained using 0.5 N HCl, ammonium oxalate, and citrate dithionite bicarbonate all underestimated the mass of BAF<sub>III</sub>.

**Table 4-2: Iron Mass Balance for Elizabeth City Fuel Farm**

| Parameter                                 | Estimated Mass (lb) |
|---|---------------------|
| Minimum advectively removed Fe II         | 13,000              |
| Maximum advectively removed Fe II         | 40,000              |
| Minimum BAF <sub>III</sub> assay estimate | 52,000              |
| Maximum BAF <sub>III</sub> assay estimate | 65,000              |
| 0.5 N HCl estimate                        | 11,000              |
| 6 N HCl estimate                          | 53,000              |
| Ammonium oxalate estimate                 | 16,000              |
| Citrate dithionite bicarbonate estimate   | 15,000              |
| Total iron estimate                       | 96,000              |

## 4.2 Performance Criteria

Performance criteria and actual performance for the BAF<sub>III</sub> assay are summarized in **Table 4-3**.

**Table 4-3: Expected Performance and Performance Confirmation Methods for BAF<sub>III</sub> Assay**

| Performance Criteria   | Expected Performance Metric (pre-demonstration) | Performance Confirmation Method   | Actual (post-demonstration)  |
|--|---|---|--|
| <b>Primary Criteria (Performance Objectives)</b><br><i>(Qualitative)</i>                           |   |   |  |
| Relationship between BAF <sub>III</sub> assay and degree of iron oxide crystallinity/surface area. | Positive association                            | Measurement of both BAF <sub>III</sub> and BET surface area for iron oxide standards with varying degrees of crystallinity and surface areas. | Generally a positive association with the exception of lepidocrocite which was expected. |

**Table 4-3: Expected Performance and Performance Confirmation Methods for BAF<sub>FeIII</sub> Assay (Cont'd)**

| Performance Criteria   | Expected Performance Metric (pre-demonstration) | Performance Confirmation Method  | Actual (post-demonstration)   |
|--|---|--|---|
| <b>Primary Criteria (Performance Objectives) (Qualitative)</b>   |   |  |   |
| Relationship between BAF <sub>FeIII</sub> assay and confirmatory analyses.                                 | Positive association                            | Multivariate statistical analysis (principal components analysis).<br><br>Loadings $\geq 0.45$ for original variables within a principal component demonstrate positive association.                         | Most of the variance in the original variables (about 43%) accounted for by two principal components. Component 2 contained the BAF <sub>FeIII</sub> variable and the BAF <sub>FeIII</sub> -relevant confirmatory analysis variables with loadings greater than 0.45. |
| Range of BAF <sub>FeIII</sub> assay relative to other analytical techniques.                               | Similar Range                                   | Comparison of analytical range to citrate dithionite bicarbonate extractable Fe, ammonium oxalate extractable Fe, total Fe, 0.5N and 6.0N HCl extractable Fe III, and to microcosm reducible FeIII with BrY. | Range was similar to or greater than all comparable methods examined. Recommended minimum BAF <sub>FeIII</sub> reporting limit is 0.1 g/kg.   |
| Sample throughput of BAF <sub>FeIII</sub> assay.   | Labor time $\leq$ similar methods               | Comparison with other methods used to characterize BAF <sub>FeIII</sub> .  | Labor time was less than or approximately the same as other methods.  |
| Versatility of BAF <sub>FeIII</sub> assay.   | Consistent performance                          | BAF <sub>FeIII</sub> assay conducted on a wide variety of soils and standards, at a wide variety of sites, and under a wide variety of environmental conditions.   | Performance was consistent with other methods used to characterize BAF <sub>FeIII</sub> .   |
| <b>Primary Criteria (Performance Objectives) (Quantitative)</b>  |   |  |   |
| Intra-laboratory precision of BAF <sub>FeIII</sub> assay based on soil and laboratory replicates.          | Absolute RPD $\leq 35$                          | Field and laboratory replicate sample collection and analyses.   | Average absolute RPD = 29.7.  |
| Inter-laboratory precision of BAF <sub>FeIII</sub> assay based on replicates analyzed by both CDM and EPA. | $-35 \geq \text{RPD} \geq 35$                   | Field and laboratory replicate sample collection and analyses. Blind standard analysis.  | Average RPD = 11.6 but difference between CDM and EPA results were not statistically significant.   |

### 4.3 Data Assessment

A general assessment of the data was included in Section 4.1 and a more detailed assessment is presented in the ESTCP Final Report (NAVFAC, 2005). In summary, the following conclusions were made based on the data:

- Intra-laboratory precision was better than the relative percent deviation (RPD) criterion of 35. This precision level is adequate for the intended use. Precision deteriorated at BAF<sub>FeIII</sub> concentrations less than the recommended 0.1 g/kg minimum reporting limit.
- Inter-laboratory precision was excellent and no statistically significant difference between CDM and EPA results was observed.
- Positive associations between the BAF<sub>FeIII</sub> assay and confirmatory analyses were observed using principal components analysis (PCA) of soil data. These results indicate the BAF<sub>FeIII</sub> assay yields results that are representative of iron bioavailability.
- An iron balance conducted on data obtained from the U.S. Coast Guard Support Station fuel farm site indicated the BAF<sub>FeIII</sub> assay yields a more robust estimate of BAF<sub>FeIII</sub> compared to common chemical extraction methods.
- Data obtained using synthetic iron oxides of varying surface area indicated the direct BAF<sub>FeIII</sub> bioassay yields results that are more representative of biological iron oxide reduction than are indirect chemical extractions.
- The BAF<sub>FeIII</sub> assay is easy to use and involves addition of weighed soil samples to pre-prepared test tubes, incubation, extraction, and measurement of reduced Fe II with a Hach test kit.

### 4.4 Technology Comparison

No other standardized and direct method exists for measurement of BAF<sub>FeIII</sub>. The data demonstrated that the BAF<sub>FeIII</sub> assay yields a more robust estimate of BAF<sub>FeIII</sub> and yields results that are more representative of biological iron reduction than chemical extraction methods.

## 5.0 Cost Assessment

ESTCP guidance states that costs should be reported in this section in the recommended Federal Remediation Technologies Roundtable (FRTR) format. However, this format is primarily suited for presenting costs associated with remedial process technologies where costs need to be broken down into categories such as capital, operational and maintenance, and life cycle costs. Costs associated with purchasing and using the bioavailable iron assays do not fall into these categories, and so the FRTR format has not been used. This alternative costing approach was used in the ESTCP Final Report (NAVFAC, 2005) that was approved by ESTCP. The subsections below have been prepared to describe all costs associated with obtaining the assays and using them to analyze soil samples that have already been collected from a given site.

### 5.1 Cost Reporting

#### Purchasing the Test Kit

The test kit is currently commercially available as the “Bioavailable Ferric Iron Assay” produced by New Horizons Diagnostics Inc. of Columbia, Maryland (NHD). Information about the kit and how to order can be found online at [www.nhdiag.com](http://www.nhdiag.com). Orders can be placed at 800-888-5015 or 410-992-9357. As of the writing of this report, the costs of the kits were:

- 1 to 11 kits: \$75 each
- 12 to 19 kits: \$60 each
- $\geq 20$  kits: \$50 each

Since the kit includes a reagent that contains bacteria which are temperature-sensitive, overnight shipping (not included in the above costs) is required. The kits contain syringes, syringe filters, hydrochloric acid, incubation and sample vials, and the lyophilized BrY inoculum.

#### Additional Supplies/Equipment

To analyze ferrous iron before and after incubation with BrY, a Hach kit is typically used. The reagent needed to run the 1,10-phenanthroline ferrous iron method (Hach Method 8146) costs \$15 for 100 Hach reagent powder pillows, or about \$18 for 25 Hach AccuVac<sup>®</sup> ampules. The Hach method also requires colorimetric analysis to quantify the ferrous iron as shown below:

1. Using a high quality spectrophotometer (Hach models are about \$2,000)
2. Using a Hach DR/800 series portable colorimeter (about \$600 to \$900)
3. Using a Hach color disc (about \$30)

The choice of which of these methods to use will depend primarily on the number of samples that are to be analyzed in the long term, whether analyses are to be performed in the field, and on the availability of the required equipment. The color disc method is semi-quantitative, and is not recommended due to its low level of accuracy relative to the other two methods. Additionally, Quantofix<sup>®</sup> Iron 1000 test strips (VWR Part No. 60787-724) may be used (without the Iron 1 reagent) to bracket the Fe II range and thus the required dilution prior to conducting the Hach assay.

If only a few samples are to be analyzed, it may be most economical to have the  $T_0$  and  $T_{30}$  extracts samples analyzed for ferrous iron by a commercial analytical laboratory. Typically, this analysis can be performed for approximately \$30 per sample (i.e., \$60/bioavailable iron sample since both the  $T_0$  and  $T_{30}$  measurements must be conducted). A small tumbler or orbital shaker is needed for the HCl extraction steps of the assay to rotate the vials and provide mixing of the soil with the acid. This item can be purchased from most lab supply companies for approximately \$250. Miscellaneous other supplies for performing the assay and ferrous iron analyses include pipettes, beakers, a small field balance (accuracy to 0.1 gram), and safety ware (gloves and glasses). An approximate cost for these supplies is \$300.

### Labor

The labor time required to perform the assay can be divided into three steps:

1. Vial  $T_0$ : Combine soil, HCl, and water. Vial  $T_{30}$ : Combine soil, water, and bioassay reagents.
2. Measure ferrous iron in Vial  $T_0$  following mixing for 48 hours.
3. After a 4-week incubation period, add HCl and measure ferrous iron in Vial  $T_{30}$  following mixing for 48 hours.

The first step takes approximately one half hour, depending on the number of samples to be run. Running the Hach kit ferrous iron analysis for Vial  $T_0$  (step 2) typically takes 1.5 hours for up to five samples – this includes time to run standards and prepare dilutions as necessary. Following the 4-week incubation period, another hour and a half would be needed for step 3 to add the HCl to Vial  $T_{30}$  and analyze for ferrous iron. If an analytical lab is used for ferrous iron analysis, the required labor would include labeling, packing, shipping the sample containers and filling out the chain of custody forms.

### Cost Example

As a costing example, consider the following scenario:

- Six soil samples are to be analyzed for bioavailable iron at a given site.
- The samples have been collected (sample collection costs were not included).
- A field technician and bench space are available to perform the extraction steps.
- Neither a spectrophotometer nor a Hach color-measuring equipment is available for the ferrous iron analysis.

Costs under this scenario are shown in **Table 5-1**.

**Table 5-1: BAF<sub>III</sub> Assay Costs**

| Item                                 | Units    | No. of Units | Unit Cost | Cost           |
|--------------------------------------|----------|--------------|-----------|----------------|
| Assay Kits                           | Each     | 6            | \$75      | \$450          |
| Ferrous Fe Analysis (commercial lab) | Sample   | 12           | \$30      | \$360          |
| Supplies                             | Lump Sum | 1            | \$100     | \$100          |
| Labor                                | Hour     | 6            | \$60      | \$360          |
| <b>Total</b>                         |          |              |           | <b>\$1,270</b> |

The unit cost per sample is thus \$212. As an alternative, commercial laboratories can also be contracted to conduct the BAF<sub>III</sub> analysis. Microseeps ([www.microseeps.com](http://www.microseeps.com)) has quoted a price of \$250 per sample for the BAF<sub>III</sub> analysis using the NHD test kit.



## 5.2 Cost Analysis

### Cost Drivers

Primary drivers are the test kit procurement cost, Fe II analysis cost, and labor cost not including soil sampling costs. Soil sampling costs will be the primary driver in cases where soil sampling is conducted solely for the purpose of BAF<sub>FeIII</sub> analysis.

While not directly related to the cost of performing the bioavailable iron kit method, the 4-week incubation period may in some circumstances result in higher indirect costs compared to a method that gives results over a 2-week period typically associated with analytical lab turnaround times. Such indirect costs need to be considered on a case-by-case basis.

If, based on the results of analyzing initial soil samples, it is determined that additional analysis is warranted, then additional costs associated with obtaining additional soil samples would be necessary. These costs would be highly site-specific and would depend on the depth of sample needed, number of samples to be collected, and site access issues.

### Sensitivity Analysis

Incremental BAF<sub>FeIII</sub> assay costs for soil sample collection are highly dependent on drilling method, depth, and sample collection frequency. For this sensitivity analysis both direct push and conventional (e.g., hollow-stem auger) methods were considered. For direct push it was conservatively assumed that 5 borings to a depth of 50 feet could be conducted per day and that 2 soil samples would be collected from each boring for BAF<sub>FeIII</sub> analysis. At a daily drilling cost of \$2,500 (\$1,500 for the driller and \$1,000 for engineering oversight) the incremental cost for drilling per sample is \$250. For conventional drilling the incremental cost was based on a unit drilling cost of \$50/foot and other parameters used for direct push. The incremental cost for drilling per sample is \$1,350 (\$1,560 - \$212). **Table 5-2** summarized the results of this sensitivity analysis demonstrating the effect of drilling costs on the total assay cost. Often other analyses including total organic carbon, cation exchange capacity, metals, grain size distribution, and USCS classification may also be conducted on the soil samples. The incremental cost is then apportioned over the various analyses.

**Table 5-2: BAF<sub>FeIII</sub> Cost Scenarios**

| Scenario   | Total BAF <sub>FeIII</sub> Assay Cost per Sample |
|--|--|
| Drilling cost not included                                   | \$210  |
| Direct push drilling included, no other analyses conducted   | \$460  |
| Direct push drilling included, 4 other analyses conducted    | \$260  |
| Conventional drilling included, no other analyses conducted. | \$1,560  |
| Conventional drilling included, 4 other analyses conducted.  | \$480  |

### DoD-Wide Savings

Standardized and cost-effective analytical technologies to support MNA and EAB efforts are necessary. The BAF<sub>Fe</sub>III assay is more costly than the current approach form BAF<sub>Fe</sub>III measurement (i.e., it is infrequently measured at the present time). However, it is anticipated that use of this method will promote more widespread acceptance and more cost-effective implementation of MNA and EAB at DoD sites.

The DoD is responsible for approximately 2,093 characterized chlorinated solvent plumes (U.S. EPA, 1997). MNA is applicable to approximately 20% of chlorinated solvent sites, or 420 of the DoD plumes (U.S. EPA, 1998). Enhanced anaerobic biodegradation (EAB) may also be applicable to many of these sites. BAF<sub>Fe</sub>III analysis has not been conducted in the past because of difficulty, lack of standardization, and cost. The BAF<sub>Fe</sub>III assay and test kit is one of several tools that can now be used to support monitored natural attenuation and enhanced anaerobic bioremediation. This test kit will benefit the DoD by making this analysis available, which will promote application of MNA and EAB at these sites. However, estimation of the DoD savings attributable to the BAF<sub>Fe</sub>III test kit alone is challenging. Nevertheless, the average cost for a pump and treat operation is \$9.8 million per site (Quinton et al., 1997). If MNA is applied to 25% of the chlorinated plumes (~100 sites) at a cost of \$1 million per site, the potential savings is significant.

### **5.3 Cost Comparison**

#### Cost Comparison

For comparison purposes, the contract analytical lab costs for conducting synthetic precipitation leaching procedure (SPLP) or toxicity characteristic leaching procedure (TCLP) analyses with zero headspace extraction (ZHE) conducted on soil samples is on the order of \$90. ZHE is required to prevent oxidation of Fe II to Fe III. The extractions would be modified to use a particular chemical extractant such as 6N HCl. However, it is important to note that extraction with 6N HCl overestimates the bioavailability of many iron oxides as shown in **Figure 4-3**. Analysis of extracts for total Fe and Fe II is on the order of \$50. Thus the total cost is on the order of \$140. There would be some labor required for labeling, packing, shipping the sample containers and filling out the chain of custody forms. This cost 30 percent less than the BAF<sub>Fe</sub> III assay cost. The cost of laboratory microcosms varies widely but typically is at least \$10,000 and can be as high as \$50,000. These costs are clearly greater than the BAF<sub>Fe</sub>III assay.

#### Cost Basis

The analytical costs listed above are based on discussions with laboratories for performing an extraction procedure similar to the toxicity characteristic leaching procedure (TCLP) as described in 40 CFR 261/SW846 Method 1311 or synthetic precipitation leaching procedure (SPLP) as described in 40 CFR 261/SW846 Method 1312. Only the extraction acid would be modified from the standard TCLP or SPLP method. The extractant would be analyzed for ferrous and ferric iron using the phenanthroline method number 3500-Fe D (Greenberg et al., 1992) with appropriate controls for acidity of the extracts.

## **6.0 Implementation Issues**

### **6.1 Cost Observations**

The unit procurement cost for the BAF<sub>Fe</sub>III test kit ranges from \$50 to \$75 depending on the number of kits purchased. The total BAF<sub>Fe</sub>III analysis cost is about \$210 not including soil collection costs. A BAF<sub>Fe</sub>III site characterization may include anywhere from 10 to 100 analyses and thus the total analytical cost would range from \$2,100 to \$21,000. This cost is generally a small fraction of the total site characterization and remediation cost. Soil sample collection will comprise the majority of the cost and thus conducting multiple analyses on collected soil samples is clearly warranted.

### **6.2 Performance Observations**

The BAF<sub>Fe</sub>III test kit met all performance objectives and criteria. The fact that the relatively low surface area iron oxide lepidocrocite had a high BAF<sub>Fe</sub>III concentration initially was an unexpected and interesting observation that supported the use of a direct bioassay approach in the test kit. This result deviated from the original hypothesis proposed for this demonstration/validation project, but was later determined to be consistent with current scientific data and theories on iron bioavailability. Therefore, the BAF<sub>Fe</sub>III test kit was demonstrated to be a reliable, precise, easy-to-use, and cost-effective assay that yields realistic and relatively robust estimates of BAF<sub>Fe</sub>III for a wide variety of soil types.

### **6.3 Scale-Up**

The BAF<sub>Fe</sub>III test kit can be used to analyze one or more samples. Scale-up is not especially relevant to this assay. However, processing of a large number of samples may warrant subcontracting the work to a commercial analytical laboratory. Larger federal laboratories or research institutions will likely be capable of easily conducting the assay in-house.

### **6.4 Other Significant Observations**

The test kit Reagent B (i.e., lyophilized strain BrY) must be kept frozen until ready for use or it will lose viability. The test kit Reagent A is stable at room temperature.

Soil sampling should be conducted with care to minimize exposure to air and oxidation of reduced iron oxides. Use of an anaerobic glove box is not considered necessary, but saturated samples should be handled quickly and packed full into jars to minimize headspace. Preferably, the assay should be initiated as quickly as possible. Maximum sample holding times for this assay have not been determined.

The BAF<sub>Fe</sub>III test kit can be procured from New Horizons Diagnostics Corporation in Columbia, Maryland. Their phone number is 1-800-888-5015 and their web address is [www.nhdiag.com](http://www.nhdiag.com).

## **6.5 Lessons Learned**

BAFeIII iron concentrations can vary laterally and vertically in impacted and background areas at a site. It is important to collect and analyze a sufficient number of samples to obtain useful results that are not obscured by heterogeneity. In general, samples should be collected from zones of greatest contaminant mass flux because it is in these zones where BAFelIII consumption will represent the most significant attenuation of contaminant mass. Samples should be collected at multiple depths along plume transects and should include several upgradient and/or cross-gradient background soil samples. Duplicate analysis of samples is recommended. While none of these recommendations are hard and fast, their intent is to dissuade the user from collecting just a few samples. Such a minimalistic approach is likely to result in less useful results.

The test kit can be used to estimate the maximum BAFelIII concentration in a soil or sediment sample. Groundwater chemistry also has a significant effect on iron bioavailability (Evans, 2000; Roden and Urrutia, 2002). Therefore, groundwater chemistry data should be considered in addition to BAFelIII assay results when making conclusions with respect to iron bioavailability.

## **6.6 End-User Issues**

Education of regulators on the importance of BAFelIII is necessary because this parameter is not commonly measured or reported. Education of regulators on this test kit will also be necessary. End users will be able to refer regulators to this Cost and Performance Report and the ESTCP Final Report (NAVFAC, 2005) when establishing the validity of this BAFelIII test kit. Currently available models that include BAFelIII as an input parameter will also promote education about and acceptance of this assay as described further in Section 6.7.

## **6.7 Approach to Regulatory Compliance and Acceptance**

Dr. John Wilson of the U.S. EPA has been a strong advocate of the need to quantify BAFelIII at contaminated sites. The EPA listed BAFelIII analysis as being under development in the Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water (U. S. EPA, 1998). Dr. Wilson identified the BAFelIII test kit as a possible solution to this need. He was thus an important partner in this ESTCP project. Measurement of BAFelIII at sites is being conducted at increasing frequency and is becoming a regular component natural attenuation and anaerobic bioremediation evaluations. An excellent example is the fate and transport model BIOPLUME IV which includes BAFelIII as an input parameter. This model is currently being beta-tested by EPA. The fate and transport model BioRedox-MT3DMS also includes BAFelIII as an input parameter (Thompson et al. 2004). The BAFelIII assay is also listed as an optional method to determine competition from iron reduction during EAB (AFCEE, 2004).

BAFelIII test kit results will be used to document natural attenuation processes and to design enhanced anaerobic bioremediation remedies. In both cases the data can be used to provide regulators with a more complete and accurate technical basis for the site remedial approach. This ESTCP Cost and Performance Report will be an instrumental component of this interaction with respect to technology validation.

## 7.0 References

- AFCEE (Air Force Center for Environmental Excellence). 2004. Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents.
- ARCADIS. 2004. 3<sup>rd</sup> Event (Quarterly, October 2003) Groundwater Monitoring Report – HRC Injection Pilot Study, North Beach Disposal Area (SWMUs 28 and 56), United States Coast Guard Support Center, Elizabeth City, North Carolina. January.
- Battelle Memorial Institute. 2000. Technology Demonstration Plan, Reductive Anaerobic Biological in Situ Treatment Technology (RABITT) Treatability Testing at the East Gate Disposal Yard Site Fort Lewis, WA.
- CDM. 2001. Technology Demonstration Plan, Development of a Dissolved Hydrogen Analyzer and a Bioavailable Ferric Iron Assay. Environmental Security Technology Certification Program Project No. CU-200009.
- Cooper, D.C., F. Picardal, J. Rivera and C. Talbot. 2000. Zinc Immobilization and Magnetite Formation via Ferric Oxide Reduction by *Shewanella putrefaciens* 200. Environ. Sci. Technol, 34:100-106.
- EA Engineering, Science and Technology. 2000. Final Technical Memorandum Preliminary Evaluation of the Natural Attenuation Process Phase II Operable Unit 8 Naval Submarine Base Bangor, Kitsap County, Washington.
- Evans, P.J. 1997. A Bioassay for Quantifying Intrinsic Bioremediation Potential of Fuel Hydrocarbons by Iron-Reducing Bacteria. Report Number AL/EQ-TR-1997-0004, DOD Contract Number F41624-96-C-0009.
- Evans, P.J. and K.A. Jones. 1999. Development of a Dissolved Hydrogen Analyzer and a Bioavailable Ferric Iron Assay. Phase II SBIR Final Report submitted to Tyndall AFB AFRL/MLQE, DOD Contract Number F41624-97-C-0005.
- Evans, P.J., K.A. Jones, C.C. Liu, and D.R. Lovley. 1999. Development of a Natural Attenuation Test Kit. In: Natural Attenuation of Chlorinated Solvents, Petroleum Hydrocarbons, and Other Organic Compounds (B.C. Alleman and A. Leeson, eds.). Battelle Press, Columbus. pp. 331-336.
- Evans, P.J. 2000. A Novel Ferric Iron Bioavailability Assay. In: Risk, Regulatory, and Monitoring Considerations (G.B. Wickramanayake et al., eds.). Battelle Press, Columbus. pp. 167-174.

Evans, P.J. and S.S. Koenigsberg. 2001. A Bioavailable Ferric Iron Assay and Relevance to Reductive Dechlorination. In: Bioaugmentation, Biobarriers, and Biogeochemistry (A. Leeson, et al., eds.). Battelle Press, Columbus. pp. 167-174.

Greenberg, A.E., L. S. Clesceri, and A.D. Eaton (eds.). 1992. Standard Methods for the Examination of Waster and Wastewater. American Public Health Association, American Water Works Association, and Water Environment Federation. Washington, D.C.

Hacherl, E.L., D. S. Kosson, L.Y. Young, and R.M. Cowan. 2001. Measurement of Iron(III) Bioavailability in Pure Iron Oxide Minerals and Soils Using Anthraquinone-2,6-disulfonate Oxidation. Environ. Sci. Technol. 35:4886-4893.

Hersman, L.E., J.H. Forsythe, L.O. Ticknor, and P.A. Maurice. 2001. Growth of *Pseudomonas mendocina* on Fe(III) (Hydr)Oxides. Appl. Environ. Microbiol., 67:4448-4453.

Lovley, D.R. and E.J. Phillips. 1987. Rapid Assay for Microbially Reducible Ferric Iron in Aquatic Sediments. Appl. Environ. Microbiol. 53:1536-1540.

NAVFAC. 2005. Final Report. Field Demonstration and Validation of a Bioavailable Ferric Iron Assay. ESTCP Project Number 200009. Naval Facilities Engineering Command (NAVFAC) Engineering Service Center. Port Hueneme, CA. Report CR-05-002-ENV.

Quinton, G.E., R.J. Buchanon, D.E. Ellis, and S.H. Shoemaker. 1997. A Method to Compare Groundwater Cleanup Technologies. Remediation, Autumn. pp. 7-16.

Roden, E.E. 2003. Fe(III) Oxide Reactivity Toward biological versus Chemical Reduction. Environ. Sci. Technol. 37:1319-1324.

Roden, E.E. and J.M. Zachara. 1996. Microbial Reduction of Crystalline Iron (III) Oxides: Influence of Oxide Surface Area and Potential for Cell Growth. Environ. Sci. Technol. 30:1618-1628.

Roden, E.E. and M.M Urrutia. 2002. Influence of Biogenic Fe(II) on Bacterial Crystalline Fe(III) Oxide Reduction. Geomicrobiol. J. 19:209-251.

Schwertmann, U., H. Kodama, and W.R. Fisher. 1986. In: Interactions of Soil Minerals with Natural Organics and Microbes (P.M. Huang and M. Schnitzer, eds.). Soil Science Society of America, Inc., Madison, Wisconsin. SSSA Special Publication Number 17. p. 237.

Thompson, A.-M., P.J. Van Geel, and W. J. Parker. 2004. Evaluating the Dissolution and Natural attenuation of Jet Fuel at a Former Tank Farm. J. Environ. Eng. Sci. 3:107-118.

U.S. Environmental Protection Agency (EPA). 1997. Clean Up the Nation's Waste Sites: Markets and Technology Trends. EPA/542/R-96/005.

U.S. Environmental Protection Agency (EPA). 1998. Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Groundwater. EPA/600/R-98/128.

U.S. Environmental Protection Agency (EPA). 2002. Contract Laboratory Program, National Functional Guidelines for Inorganic Data Review. EPA 540-R-01-008.

U.S. Geological Survey and Southern Division, Naval Facilities Engineering Command. 1999. Monitoring Plan for Natural Attenuation Remediation, Waste Water Treatment Plant, Pensacola, Florida.

Wilson, J.T., Cho, J.S., Wilson, B.S. and J.A. Vardy. 2000. Natural Attenuation of MTBE in the Subsurface under Methanogenic Conditions. EPA/600/R-00/006.

## 8.0 Points of Contact

### 8.1 ESTCP Program Office

Andrea Leeson  
SERDP  
901 N. Stuart St., Suite 303  
Arlington, VA 22203-1853  
Phone: (703) 696-2118  
Fax: (703) 696-2114  
Email: [Andrea.Leeson@osd.mil](mailto:Andrea.Leeson@osd.mil)

Scott Dockum  
HydroGeoLogic, Inc.  
1155 Herndon Parkway, Suite 900  
Herndon, VA 20170  
Phone: (703) 326-7808  
Fax: (703) 478-0523  
Email: [sdockum@hgl.com](mailto:sdockum@hgl.com)

### 8.2 Project Management

Carmen A. Lebron (Principal Investigator)  
NFESC  
Restoration Development Branch  
Code ESC411  
1100 23<sup>rd</sup> Avenue  
Port Hueneme, CA 93043-4370  
Phone (805) 982-1616  
Fax: (805) 982-4304  
Email: [carmen.lebron@navy.mil](mailto:carmen.lebron@navy.mil)

Barbara Sugiyama  
NFESC  
Restoration Development Branch  
Code ESC411  
1100 23<sup>rd</sup> Ave.  
Port Hueneme, CA 93043  
Phone: (805) 982-1668  
Fax: (805) 982-4304  
Email: [barbara.sugiyama@navy.mil](mailto:barbara.sugiyama@navy.mil)

### 8.3 CDM Staff

Patrick Evans (Co-Principal Investigator)  
CDM  
11811 NE 1<sup>st</sup> Street, Suite 201  
Bellevue, WA 98005-3033  
Phone: (425) 453-8383  
Fax: (425) 646-9523  
Email: [evanspj@cdm.com](mailto:evanspj@cdm.com)

Roger Olsen  
CDM  
1331 17<sup>th</sup> Street  
Suite 1200  
Denver, CO 80202  
Phone: (303) 298-1311  
Fax: (303) 293-8236  
Email: [olsenrl@cdm.com](mailto:olsenrl@cdm.com)

Rick Chappell  
CDM  
1331 17<sup>th</sup> Street  
Suite 1200  
Denver, CO 80202  
Phone: (303) 298-1311  
Fax: (303) 293-8236  
Email: [chappellrw@cdm.com](mailto:chappellrw@cdm.com)



## 8.4 Partners

Eric Weber  
EPA  
960 College Station Road  
Athens, GA 30605-2700  
Phone: (706) 355-8224  
Fax: (706) 355-8202  
Email: [weber.eric@epa.gov](mailto:weber.eric@epa.gov)

John Drexler  
Geology Department  
University of Colorado  
Benson Earth Science building  
Room 125  
2200 Colorado Avenue  
Boulder, CO 80309  
Phone: (303) 492-5251  
Fax: (303) 492-2606  
Email: [drexlerj@spot.colorado.edu](mailto:drexlerj@spot.colorado.edu)

John Wilson  
EPA  
Kerr Lab Road  
Ada, OK 74820  
Phone: (580) 436-8534  
Fax: (580) 436-8534  
Email: [Wilson.Johnt@epamail.epa.gov](mailto:Wilson.Johnt@epamail.epa.gov)

Thomas DiChristina  
School of Biology  
Cherry Emerson Building  
Ferst Drive  
Georgia Institute of Technology  
Atlanta, GA 30332-1230  
Phone: (404) 894-8419  
Fax: (404) 894-0519  
Email: [thomas.dichristina@biology.gatech.edu](mailto:thomas.dichristina@biology.gatech.edu)

---

Signature of Project Lead

---

Date